REMARKS

This application has been carefully reviewed in light of the Office Action of September 20, 2004. By way of this amendment, claim 1 has been amended. Applicant requests further review and reconsideration in light of the following remarks.

Claims 1-4 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite. In response thereto, Applicant has amended claim 1. Specifically, claim 1 has been rewritten to delete the phrase "preferably". In light of this amendment, it is submitted that the claims are definite and the section 112 rejections should be withdrawn

Claim 3 has been rejected under 35 U.S.C. 102(b) as being anticipated by ATCC Bacteria and Bacteriophages, 19th Ed., P. Pienta et al, eds., pp. 72 and 301, American Type Culture Collection, Rockville, MD, 1996 ("ATCC"). This rejection is respectfully traversed.

The examiner has stated that "[c]laim 3 as amended reads on a biologically pure culture of Rhizobium japonicum, because the comprising language of claim 1 encompasses the steps of purifying the microorganism. Thus, the resulting product is an inoculating composition for use with leguminous plants containing just R. japonicum."

Claim 3 recites "[a] concentrated inoculating composition for use with leguminous plants, produced by the method described in claim 1." Thus, the inoculating composition is the result of carrying out all of the steps recited in claim 1. These steps include the addition of maltose saccharide and potassium sorbate to the R. japonicum, as well as a culture medium. This claim, while containing an open-ended "comprising" transition phrase, certainly does not read on an inoculating composition containing just R. japonicum. In fact, the Examiner has noted, at page 6 of the Office Action, that the composition recited in claim 3 "may also contain [in fact does contain] components or by-products of the culture medium or maltose or lactose or potassium sorbate."

Accordingly, it is submitted that the ATCC reference clearly fails to disclose every element of claim 3 and the rejection should be withdrawn.

Claim 3 has been rejected under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent No. 3,168,796 (Scott et al.), Kuykendall et al., and Bergey's Manual, in view of U.S. Patent 5,750,402 (Guri et al.) and U.S. Patent 4,755,468 (Jung et al.) This rejection is respectfully traversed.

The Applicant does not dispute that Rhizobium may be grown using standard procedures well known in the art and as taught by Scott et al. and Kuykendall et al.

Scott et al. discloses a dry inoculant composition of special substantially moisture-free dormant character and powdery carriers or intermediates, saying that the composition might contain special additives, such as, for example, sugars which provide a food supply for the bacteria and which also give some evidence of serving a protective function for the bacteria (see column 3, lines 47 to 52).

But when Scott et al. explains how to grow the bacteria (see column 5, line 13), it does through an example, disclosing that the sterile media and water mixture (i.e. the culture medium) suitably consists of about 1% sucrose or other suitable carbohydrates as a carbon source. It means that the sugar is added as a part of the culture medium, before incorporating the bacteria for growing. So Scott et al. adds sugar to the culture medium, and then adds the bacteria, unlike the present invention.

Finally, at column 5, last paragraph, Scott et al. indicates that after subjecting the bacteria to a series of treatments to convert it into a substantially moisture-free state (see column 5, lines 37 to 55 and lines 73 to 76) (with or without a later washing), a quantity of a nutrient material can be added, examples of which are mono or di -saccharides such as sucrose and glucose. This means that these nutrient materials are for a different purpose than the present invention which is to protect the bacteria's membrane.

According to Scott et al., saccharides are added not immediately after the bacteria has multiplied, as in the present invention, but after the treatment to convert the bacteria into a substantially moisture-free state (see column 5, lines 37 to 55). Scott et al thus

teaches an additional step between the bacteria multiplication and the addition of the saccharides, which can damage the bacteria's membrane.

Scott et al. states that "the bacterial suspension is subjected into a substantially moisture-free state without killing them. Sublimation of the water from the aerobic Rhizobium has been discovered to be entirely effective, but must be accomplished with care in order to avoid killing the Rhizobium." See column 5, lines 37-38. This means that after the multiplication step of Scott et al, when the water is sublimated out, the bacteria is subject to destruction. Accordingly, the adding of saccharides after that step can not have any protective effect if the bacteria are already dead. Therefore, the order of addition of the elements is essential to avoid the risk of obtaining a useless inoculant composition with dead bacteria.

The present invention adds the saccharides together with the potassium sorbate to the bacteria culture (not to the culture medium), treated in the way described in the invention, because this is the only way to inhibit the bacteria multiplication to the correct degree as well as protect the bacteria membrane, with the result that the bacteria culture survives over 18 months.

To obtain this result, it is necessary to add saccharides together with the potassium sorbate treated in the way described in the specification to the bacteria culture (not to the culture medium), immediately after the multiplication of Rhizobium is finished, and without any intermediate steps as described in Scott et al.

Scott et al. states that after having added the saccharides after the sublimation process, there is not a way to prove the bacteria culture is alive, since it is stated that "quantities of bacteria in our composition are given in weight measurements, without discussion of bacterial count". This means that the existing bacteria in Scott et al. are counted by the weight the composition has achieved when the bacteria multiplied at the multiplication step. But, since Scott et al. does not add maltose after the multiplication, but after the sublimation process, Scott et al. can obtain a composition which could increase

its weight by multiplication, and then be destroyed at the sublimation process. It is not of practical benefit to obtain a composition with a large quantity of dead R. japonicum.

The present invention, by adding powdered maltose saccharide and liquid maltose saccharide together with potassium sorbate, all treated in the way described in claim 1 (i.e. immediately after the bacteria multiplication process), protects bacteria membrane, because such elements act like growing inhibitors and stabilizers which keep the bacteria alive and lethargic.

Without such stabilization, the culture could not to survive more than thirty days. For that reason, following Scott et al., it would be possible to keep a composition during 1, 2, 3 years or more, but without knowing the bacterial count, one would never know if the composition has at least one bacteria alive.

At any rate, if the sublimation step does not destroy all of the bacteria, the addition of saccharides without potassium sorbate and without both of them treated in the way described in claim 1, will tend to destroy the Rhizobium culture, because the bacteria will use the saccharides as nutrients, multiplying indefinitely until death, because the bacteria culture would not have the potassium sorbate treated in the way described in claim 1 which has functions to inhibit an excessive bacteria growing and, together with the saccharides, keep the Rhizobium lethargic.

With the present invention, the bacteria culture can last from 18 to 24 months alive, without altering their properties.

As the Examiner has noted, Kuykendall et al. teaches a Rhizobium culture medium and sterilized sugars, including maltose, as well as a method for making for this medium. However, claim 3 does not recite solely a culture medium, with or without sugars, but an inoculant composition obtained by the method described in claim 1.

Kuykendall et al. uses the ten carbon sources mentioned by the examiner (page 516, 2nd. Paragraph.) as ingredients nutrients of the culture medium. On page 512 "Media", it is stated that when "a medium containing only a principal carbon source was

desired, the sugar was autoclaved". Also at page 512 under the heading "Planting on various carbohydrate-supplemented plates", it is noted that the "sugar solutions" are added as ingredients of the culture medium, where later the bacteria will be multiplied and take such sugars as nutrients. It is stated that "After the solidification, the plates were spread with 0.1 ml portions of 10-5 and 10-6 dilutions of mid-log phase R. Japonicum". That is, the bacteria is later incorporated to multiply it. In any case, if it were desired to use the culture medium of Kuykendall et al., (or any other culture medium) with the present invention, one would have to wait till the bacteria multiplication is finished and then add the saccharides together with potassium sorbate, all treated in the way described in claim 1, contrary to what is taught in Kuykendall et al.

Kuykendall et al., at page 516, 2nd Paragraph, makes the culture (not the culture medium) with a strain L1-110 in a culture medium made of "agar media supplemented with different sugars ...carbon sources tested were...maltose". That means that Kuykendall et al. does not add the maltose to the bacteria culture, but to the culture medium. Kuykendall et al. makes the culture medium with maltose and other elements. The present invention does not change such culture medium nor any other existing culture medium, but teaches a method of how to continue processing once the bacteria multiplication has been obtained.

The order of addition of elements to the claimed composition is essential, because by adding to the bacteria culture (not to the culture medium) powdered maltose saccharide, liquid maltose saccharide together with potassium sorbate, all treated in the way described in claim 1, the bacteria's membrane of the multiplied bacteria will be protected, inhibiting their later growth obtaining a useful inoculant, which will last from 18 to 24 months.

If the culture medium is prepared with maltose and any other components but without the addition of the claimed elements after bacteria multiplication, the Rhizobium won't last more than 30 days without cold storage.

The examiner has stated that Guri et al. teaches that potassium sorbate is a known microbicide for use with plant growth medium, and that Jung et al. teaches that fungicides may be added to a plant growth medium without altering the bacterial cells.

Guri et al. is related to the culture of plant cells (see column 10, line 13), which is very different than the present invention based on bacteria. Guri et al. discusses different elements (column 1, "Background of the invention") which are added to the culture medium to destroy bacteria growth. At line 46 of column 1, it states that "unfortunately, such culture media also provide a rich mixture of nutrients which can support the rapid growth of bacteria and fungi. Once these contaminants are establish in the culture they usually grow quickly". Then, it says (see column 2, line 7 to 11) "it would be useful to provide a chemical agent that reduces o prevents the microbial contamination of plant tissue culture media..."

As can be seen at the heading 2.2 ANTIMICROBIAL AGENTS IN PLANT TISSUE CULTURE (column 2), such chemical agents are also used in culture mediums. In one of the parts in which Guri et al. describes in which stage the antimicrobial agent is added (column 2, line 61 refers to DPC "to chemical sterilize nutrient media for plant cell cultures").

At column 3, lines 11 to 15, Guri et al. refers to "culturing a tissue slice on culture medium containing fungicide". Then, at next paragraph, it mentions that the "use of allylisothiocyanate (CH2= CHCH2=C=S) as a sterilizing agent in plant culture media."

Then, in column 3, lines 23, it is stated that "tests indicated that when these agents were included in the incubation media..."

Thus, Guri et al. consistently teaches the addition of the antimicrobial agent in the culture medium, as an ingredient thereof, for the purpose of combating contaminants like bacteria (such as Rhizobium).

Contrary to the teaching of Guri et al., the present invention does not use fungicides at all as parts of the culture medium nor added to the same, even during the bacteria multiplication process. The fungicide potassium sorbate is not used as a fungicide in the

claimed process. This is because before adding the potassium sorbate and the saccharides, the bacteria culture must not have any contaminants. The most minimum contamination will destroy the composition, and the incorporation of potassium sorbate could not fix the situation, losing the all the product. Furthermore, the potassium sorbate acts as a fungicide when there is an acid PH, and the culture medium has neutral PH. So the potassium sorbate is not a fungicide when it is added to a culture medium.

At any rate, the present invention does not add potassium sorbate alone, but together with powdered maltose saccharide, and liquid maltose saccharide, all treated in the way described in claim 1. Otherwise, the lack of saccharides will leave the bacteria membrane unprotected. Now, if after (not before or at the same time) the culture multiplication, potassium sorbate is added to the culture (not to the culture medium) together with powdered maltose saccharide, and liquid maltose saccharide, all treated in the way described in claim 1, these elements will stabilize the bacteria The order of addition of the composition's elements to the composition is essential, because addition of the potassium sorbate as one of the elements of the culture medium or during the bacteria multiplication process, would reduce or prevent the multiplication before the ideal time.

It is true that Jung et al. teaches that a fungicide may be added to a particular kind of plant culture medium. However, this is a result of the drying process taught by Jung et al. At column 7, lines 28-30, it is stated that "[a]t water activities of less than 0.1, there is no loss of the microorganism due to the presence of the fungicide". In contrast, the present invention provides a liquid inoculating composition with live bacteria which are not affected by the fungicide.

The present invention is a combination in an essential order, in order to modify the properties of the constituent elements to obtain an unexpected result. So the order of addition of the composition's elements to the composition is essential, because if the elements are added in a different way, it would be impossible to obtain the expected

results. These method steps in the claimed order result in a novel inoculant composition containing live, protected R. japonicum with a long shelf life at ambient temperatures.

Accordingly, it is submitted that the combination of references fails to teach every element of claim 3 and the rejection should be withdrawn.

Claim 4 has been rejected under 35 U.S.C. 103(a) as being unpatentable over by U.S. Patent No. 3,168,796 (Scott et al.), Kuykendall et al., and Bergey's Manual, in view of U.S. Patent 5,750,402 (Guri et al.) and U.S. Patent 4,755,468 (Jung et al.), and further in view of U.S. Patent 5,695,541 (Kosanke et al.) or U.S. Patent 5,586,411 (Gleddie et al.) This rejection is respectfully traversed.

Claim 4 depends from claim 3 and is thus believed to be allowable for the reasons stated above.

Furthermore, It is true that Kosanke et al. teaches that peat has been used in the prior art as a Rhizobium culture carrier. Indeed, many inoculant compositions with peat as a carrier have been commercialized in the market for years.

However, the prior art has not used peat as one of the ingredients to prepare the culture medium. According to Kosanke et al., in the prior art, it is usual "mixing an active, living rhizoid bacterial culture with a carrier such as humus or peat." (see column 1, line 28 to 30). Notice that Kosanke et al. does not say "culture medium" but "bacterial culture"

In this sense the usual process followed in the prior art is this: first, the culture medium is prepared, the bacteria is multiplied in that culture medium, to achieve a bacterial culture. Then, such bacterial culture was mixed with peat. The logic of the prior art is this: bacterial culture + peat = inoculant composition.

The present invention does not add peat to a bacterial culture already finished, but it uses peat as one of the ingredients which are added to prepare the culture medium, where the bacteria will be added later to produce the bacterial culture or Rhizobium multiplication.

The present invention uses peat as one of the ingredients to be used in the preparation of the culture medium. Thus, the present invention's logic is this: usual ingredients + peat = culture medium.

The order of addition is essential, because if peat is added to a bacterial culture, per Kosanke et al., one will obtain a better Rhizobium viability and a better nodulation, but the acidity of the peat itself and its different pH versus the bacterial culture's PH in which it was incorporated does not allow an optimum absorption of the Rhizobium Japonicum bacteria. That is the reason why the results are so different, as shown in table I, II and II of the specification.

Gleddie et al. refers to peat as a carrier, and not as an culture medium's ingredient. For that reason, Gleddie et al. does not teach using peat as one of the ingredients to prepare the culture medium, as presently claimed. The bacterial culture in Gleddie et al. is the result of multiplying the Rhizobium in the culture medium. Adding of peat at Gleddie et al. is not to the culture medium but to the bacterial culture. Gleddie et al. states that "a suspension of Rhizobium sp. cells is added using aseptic technique, to the peat composition" (see column 7, lines 35 to 38). In consequence, the Gleddie et al. method consists in adding bacteria directly to the peat, and not adding peat to other ingredients to obtain a "culture medium", as in the present invention.

Again, the order of addition is essential, because a simple modification will not obtain the expected results.

The present invention is not made with peat which is sterilized with the culture medium, but the culture medium is made, between other components, with peat treated in the way described in the specification. The peat cannot be differentiated from the culture medium, because the culture medium is formed with peat as one of its parts. Accordingly, it is submitted that the combination of references fails to teach every element of claim 4, and the rejection should be withdrawn.

Applicant notes with appreciation that claims 1 and 2 are allowed.

In view of the above, it is submitted that the claims are in condition for allowance. Reconsideration of the objections and rejections is requested. Allowance of claims 1-4 at an early date is solicited.

Respectfully submitted,

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